A mannose-functionalized pillar[5]arene-based supramolecular fluorescent probe for real-time monitoring of gemcitabine delivery to the cancer cells

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Supplementary Information

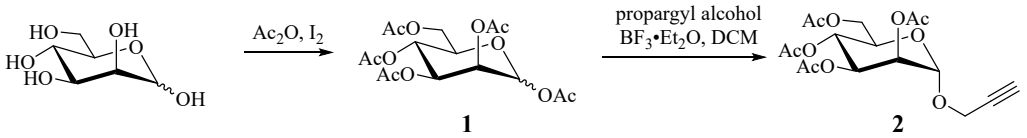
1. General information

All reagents were purchased from commercial suppliers and used without further purification unless specified. Gemcitabine (GEM) was purchased from Leyan (Shanghai, China). NMR spectra were recorded on a Bruker 500 MHz Spectrometer, with working frequencies of 500 MHz for ^{1}H and 125 MHz for ^{13}C nuclei. SEM image was obtained using a Nano SEM-450 (FEI, U.S.A.) with an accelerating voltage of 10.0 kV. TEM image was obtained by TECNAI G2 SPIRIT BIO (FEI, U.S.A.). UV-vis spectra were recorded with Shimadzu 1750 UV-visible spectrophotometer (Japan) at 298 K. Dynamic light scattering (DLS) were carried out on a ZEN3600 instrument (Malvern Instruments Limited, UK). Human breast cancer cells MCF-7, human liver cells HL7702, and human renal epithelial cells 293T were obtained from KeyGEN BioTECH Co. (Nanjing, China). Cell culture was carried out in an incubator with a humidified atmosphere of 5 % CO_2 at 37℃. HRMS (High Resolution Mass Spectrometer) analysis was performed with an AB SCIEX LC-30A-Triple TOF 5600+.

2. Live subject statement

All experiments were performed in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects of World Health Organization, and approved by the Northwest A&F University Animal Care Committee.

3. Synthesis and characterizations



Scheme S1. Synthetic route for alkyne-substituted mannose

Synthesis of compound 1[S1]. Mannose (2 g, 11.1 mmol) and iodine (0.044 g, 0.17 mmol) were added into a 50 mL round bottom flask and dissolved in acetic anhydride (20 mL, 219 mmol) at room temperature. After solids were dissolved completely, the aqueous solution was extracted with dichloromethane three times. The organic phase was washed with saturated Na_2CO_3 and $NaHCO_3$ solutions, then dried over anhydrous Na_2SO_4 and evaporated under vacuum to afford compound **1**.

Synthesis of compound 2 [S1]. Compound **1** (1.1 g, 2.82 mmol), propargyl alcohol (683 μL, 11.28 mmol), and $BF_3 \cdot Et_2O$ were stirred to dissolve in dry dichloromethane (30 mL) at 0°C for 48 h. After 50 mL dichloromethane was added, the resulting solution was washed with 20% Na_2CO_3 solution (2 × 50 mL), dried over anhydrous Na_2SO_4 and evaporated under vacuum to remove solvent. The crude product obtained was purified by silica column chromatography using petroleum ether/ethyl acetate (2:1, *v*/*v*) to afford compound **2** as a white solid (828 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 5.35 (dd, J = 10.0, 3.3 Hz, 1H), 5.31 (d, J = 10.8 Hz, 1H), 5.29-5.27 (m, 1H), 5.03 (s, 1H), 4.29 (dd, J = 12.2, 3.8 Hz, 3H), 4.12 (dd, J = 12.3, 2.2 Hz, 1H), 4.05-4.00 (m, 1H), 2.48 (s, 1H), 2.17 (s, 3H), 2.11 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H).

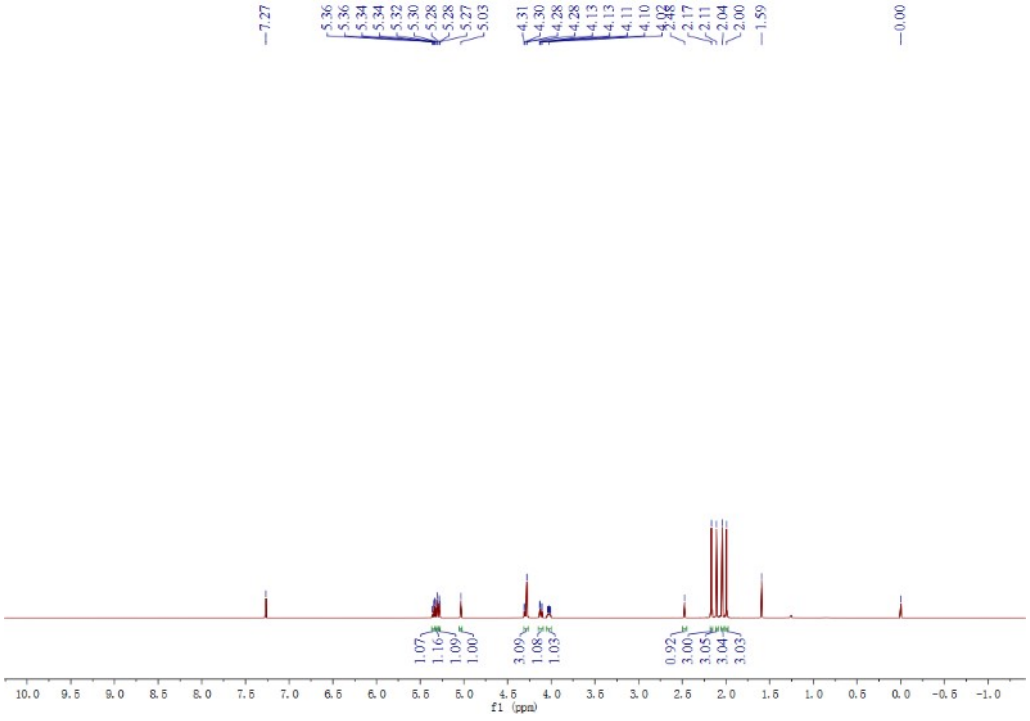
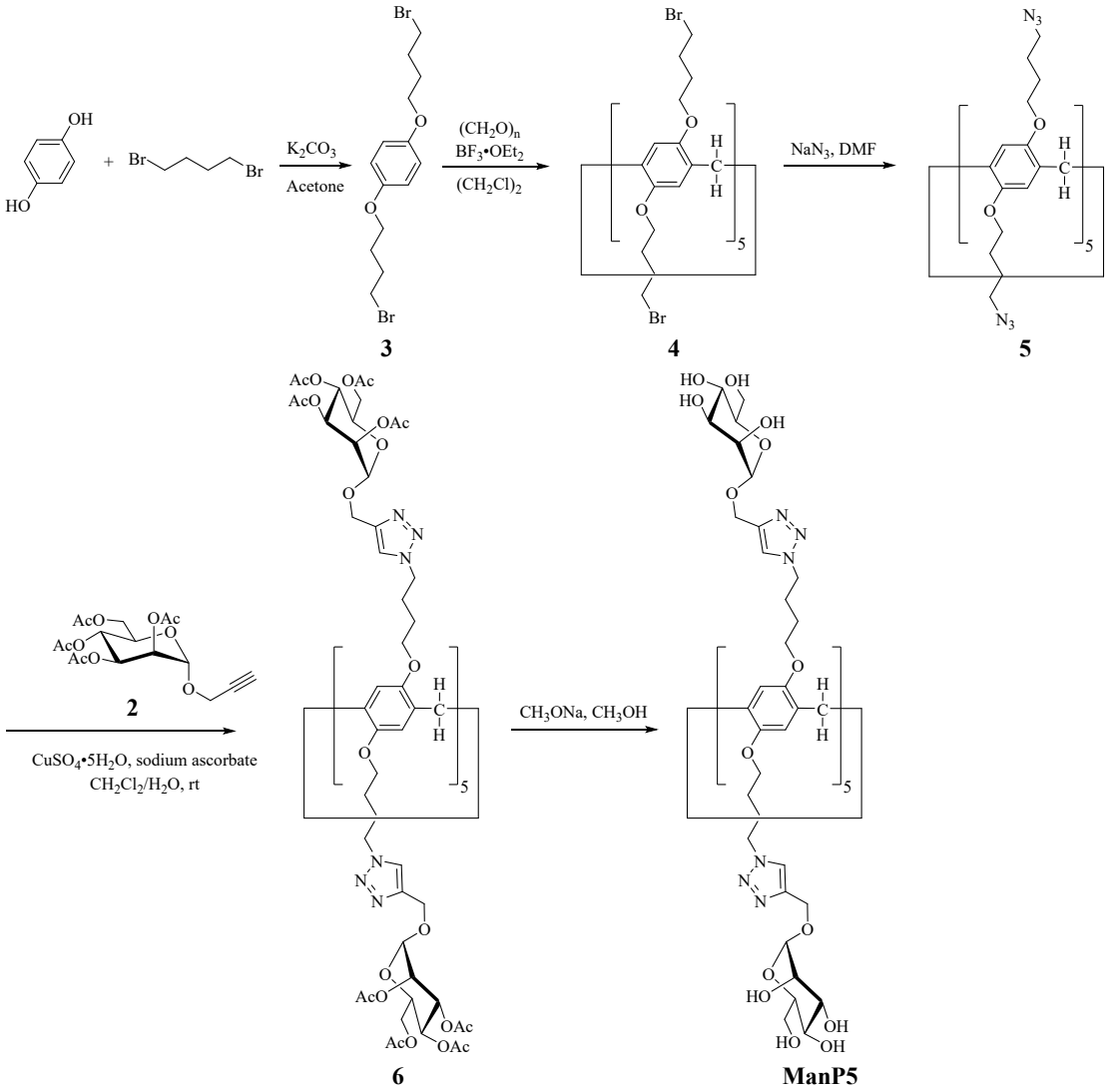


Fig. S1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298K) of compound **2**



Scheme S2. Synthetic route for mannose-modified pillar[5]arene (**ManP5**)

Synthesis of compound 3[S2]. Under nitrogen atmosphere, hydroquinone (5.5 g, 50 mmol) and 1,4-dibromobutane (41.4 g, 300 mmol) were dissolved in acetone (300 mL), followed by adding of K_2CO_3 (43.2 g, 200 mmol). The mixture was stirred at reflux for 48 h and then was poured into ice water to quench. After filtration, the residue was dissolved in dichloromethane and washed with diethyl ether. The organic phase was combined and dried over anhydrous $MgSO_4$. After removal of the solvent under reduced pressure, the crude product obtained was purified by silica column chromatography using petroleum ether/dichloromethane (1:1, *v*/*v*) to afford compound **3** as a white solid (18 g, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.81 (s, 4H), 3.95 (t, 4H, J = 6.1 Hz), 3.50 (t, 4H, J = 6.7 Hz), 2.09 (m, 4H), 1.94 (m, 4H).

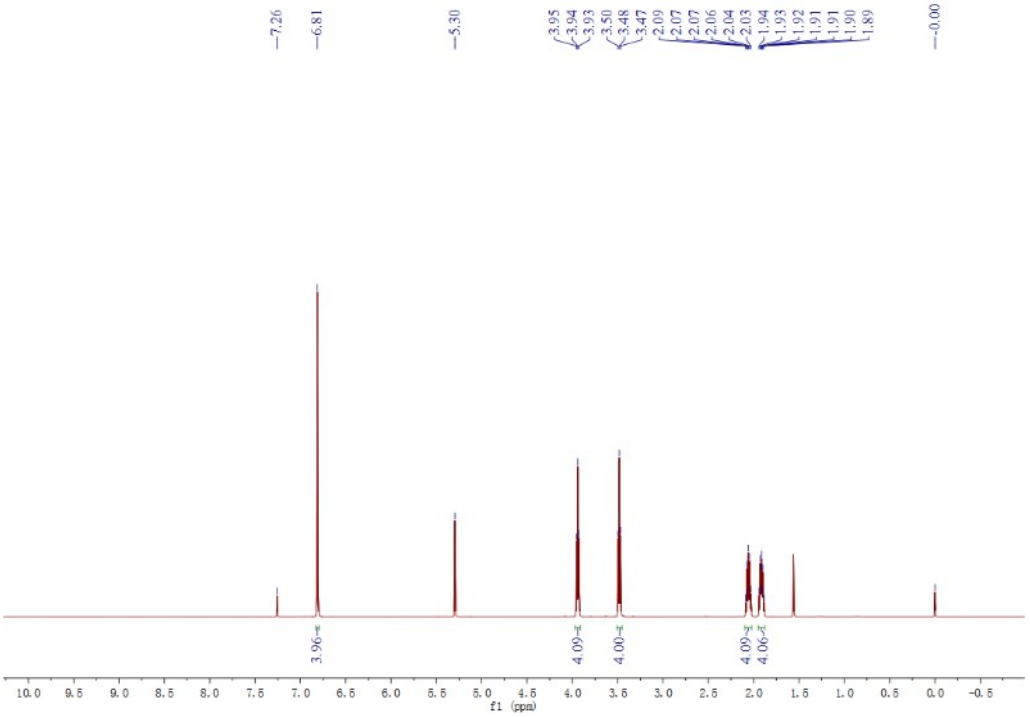


Fig. S2. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298K) of compound **3**

Synthesis of compound 4[S2]. Compound **3** (641.7 mg, 1.69 mmol) and paraformaldehyde (65.8 mg 0.73 mmol) were stirred and dissolved in 1,2-dichloroethane (20 mL) at room temperature for 10 min. After boron trifluoride etherate (0.271 g, 0.2 mmol) was added, the mixture was further stirred for another 2 h and quenched with ice water, then the mixture was separated and washed with water. The solvent was removed under vacuum and then purified by silica column chromatography using petroleum ether/dichloromethane (1:1, *v*/*v*) to afford compound **4** as a white solid (265 mg, 40%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.80 (s, 10H), 3.92 (t, J = 5.8 Hz, 20H), 3.75 (s, 10H), 3.44 (t, J = 6.5 Hz, 20H), 2.04 (m, 20H), 1.91 (m, 20H).

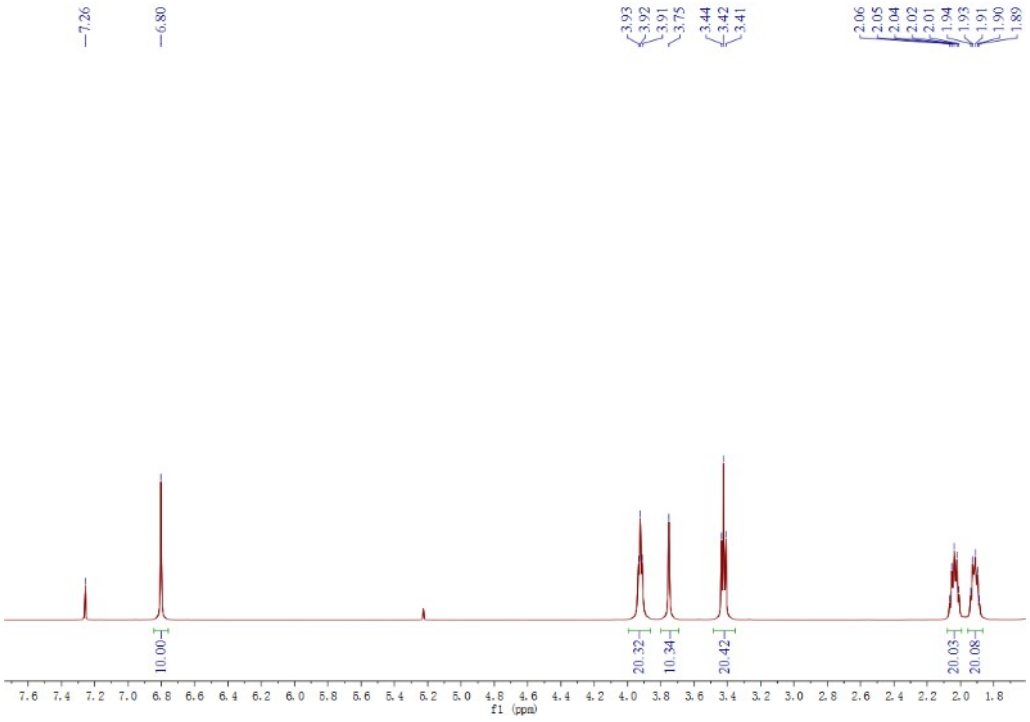


Fig. S3. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298K) of compound **4**

Synthesis of compound 5[S2]. Compound **4** (101 mg, 0.05 mmol) was dissolved in *N*, *N*-dimethylformamide (14 mL), then NaN_3 (36 mg, 0.55 mmol) was added. The mixture was heated to 80°C for 12 h. The mixture was cooled to room temperature, and then 30 mL dichloromethane was added. The resulting mixture was washed with H_2O (3 × 15 mL) and saturated NaCl (3 × 15 mL), then was dried over anhydrous Na_2SO_4. The solvent was removed under vacuum to give a white solid (78.5 mg, 96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.78 (s, 10H), 3.90 (t, J = 5.8 Hz, 20H), 3.75 (s, 10H), 3.29 (t, J = 6.8 Hz, 20H), 1.85-1.72 (m, 40H).

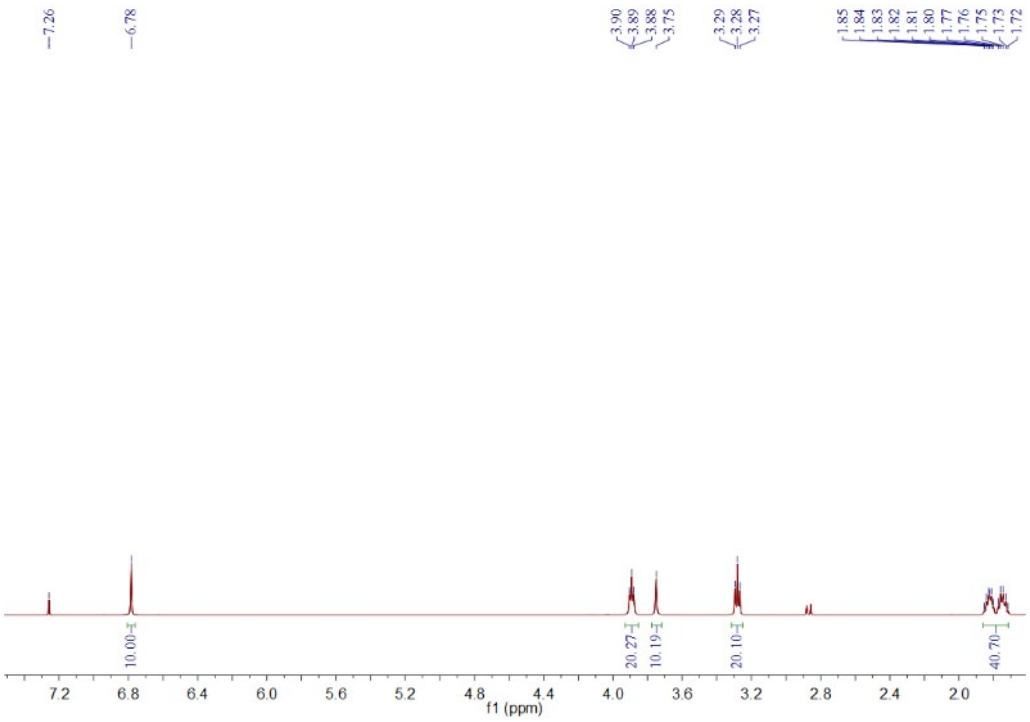


Fig. S4. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298K) of compound **5**

Synthesis of compound 6[S2]. Compound **5** (206 mg, 0.13 mmol), Compound **2** (552 mg, 1.43 mmol) and $CuSO_4 \cdot 5H_2O$ (3.3 mg, 0.013 mmol) were dissolved in CH_2Cl_2/H_2O (1:1, 6 mL), followed by adding of sodium ascorbate (7.9 mg, 0.04 mmol) under nitrogen atmosphere. The resulting mixture was stirred at room temperature for 24 h. After water was added, the aqueous layer was extracted with dichloromethane. The organic phase was combined and dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by silica column chromatography using dichloromethane /methanol (96:4, *v*/*v*) to afford a bright yellow solid **6** (587 mg, 83 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.72 (s, 10H), 6.75 (s, 10H), 5.32-5.27 (m, 30H), 5.22 (s, 10H), 4.97 (s, 10H), 4.83 (d, J = 12.2 Hz, 10H), 4.66 (dd, J = 12.2, 3.5 Hz, 10H), 4.43 (d, J = 6.8 Hz, 20H), 4.30 (dd, J = 12.2, 4.7 Hz, 10H), 4.14-4.07 (m, 20H), 3.93 (s, 10H), 3.80 (d, J = 8.2 Hz, 10H), 3.71 (s, 10H), 2.13 (s, 40H), 2.10 (s, 30H), 2.03 (s, 30H), 1.97 (s, 30H), 1.81 (s, 20H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 170.80, 170.15, 170.06, 169.81, 149.83, 143.54, 128.54, 123.35, 96.98, 69.49, 69.21, 68.78, 67.79, 66.10, 62.42, 61.02, 53.85, 53.54, 50.21, 27.40, 26.88, 20.99, 20.90, 20.82, 20.80. HRMS: m/z 5468.6670 $[M + Na]^+$.

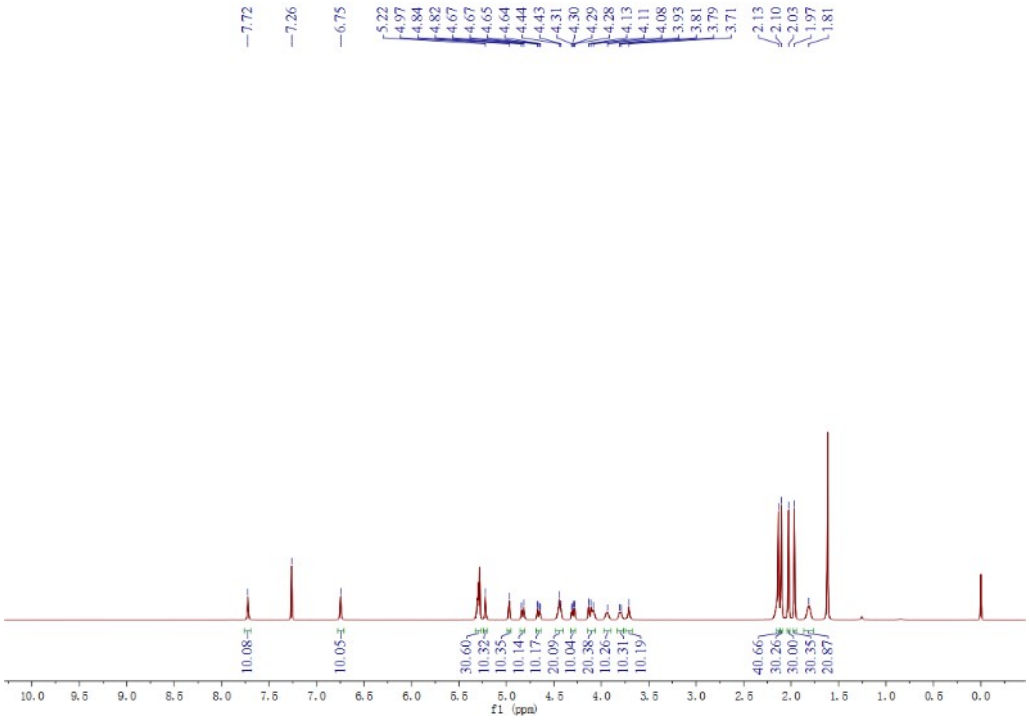


Fig. S5. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298K) of compound **6**

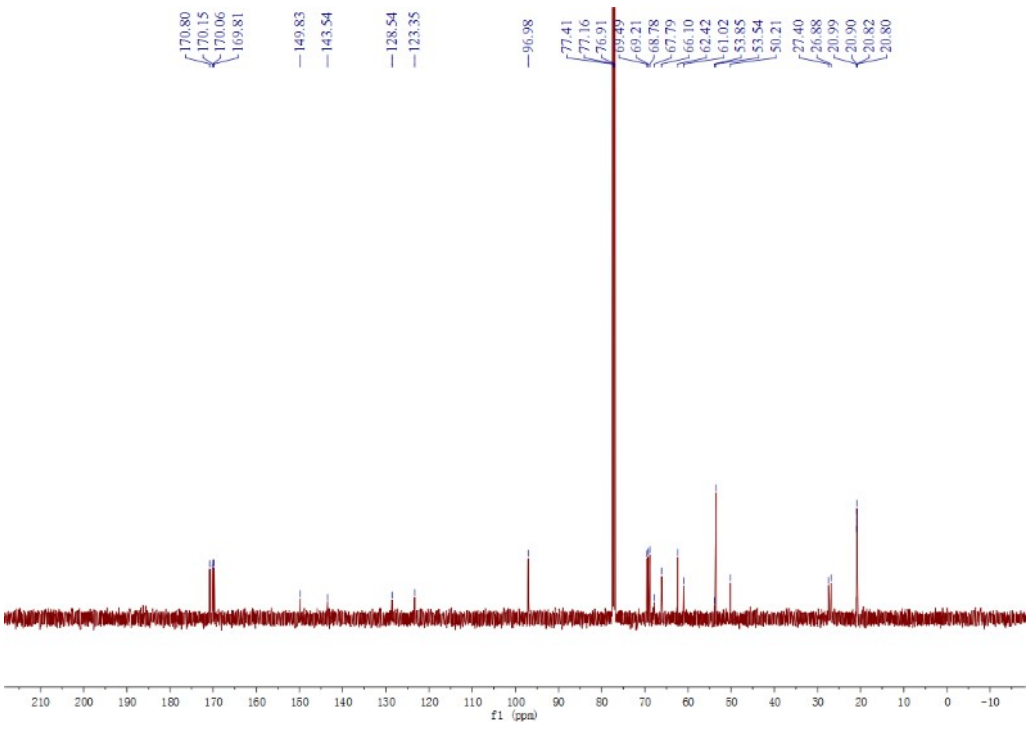


Fig. S6. ^{13}C NMR spectrum (125 MHz, $CDCl_3$, 298K) of compound **6**

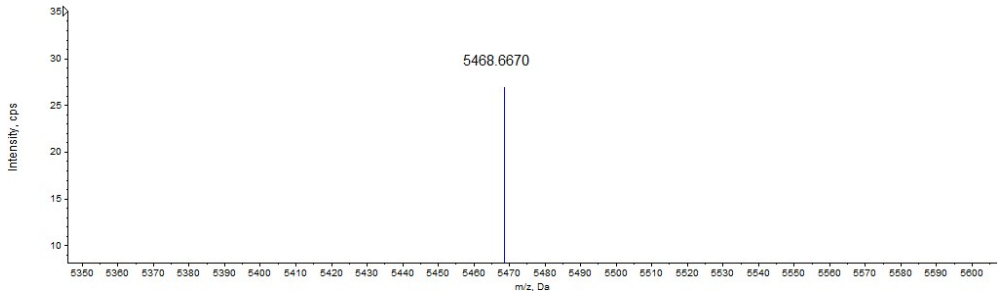


Fig. S7. ESI-MS spectrum of compound **6**

Synthesis of compound ManP5[S3]. Compound **6** (582.4 mg, 0.1 mmol) was stirred to dissolve in sodium methanolate solution (7 mL, 0.15 M) under nitrogen atmosphere. The resulting mixture was stirred at room temperature for 12 h. After filtration at the end of the reaction, the obtained solid was washed with methanol. The solvent was removed under vacuum to give a white solid **ManP5** (211 mg, 52%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.15 (s, 10H), 6.75 (s, 10H), 4.73 (s, 20H), 4.66 (d, J = 12.2 Hz, 10H), 4.50 (d, J = 12.1 Hz, 20H), 4.45 (t, J = 6.6 Hz, 20H), 3.97 (s, 10H), 3.69 (d, J = 11.3 Hz, 20H), 3.60 (d, J = 22.5 Hz, 20H), 3.51-3.37 (m, 60H), 2.07 (s, 20H), 1.73 (s, 20H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 149.47, 143.99, 142.70, 128.45, 124.57, 114.63, 105.00, 99.54, 74.60, 71.39, 70.67, 67.81, 67.47, 61.75, 60.64, 59.62, 55.39, 49.80, 49.09, 27.30, 26.89. HRMS: m/z 3785.9442 [M + Na]+.

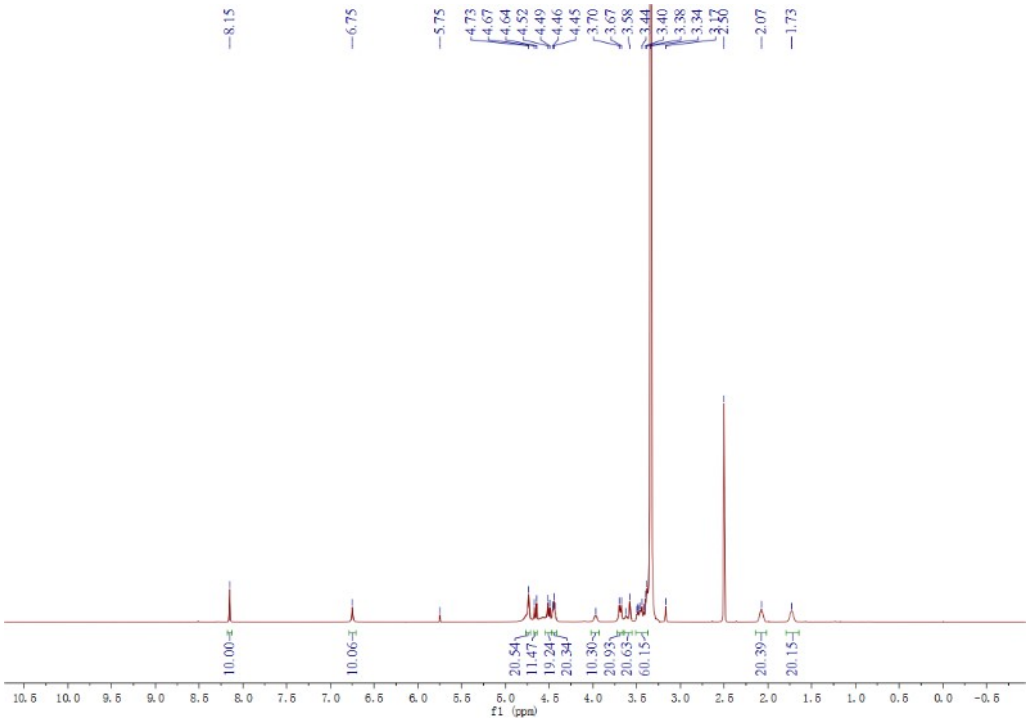


Fig. S8. ^{1}H NMR spectrum (500 MHz, DMSO-d_6, 298K) of compound **ManP5**

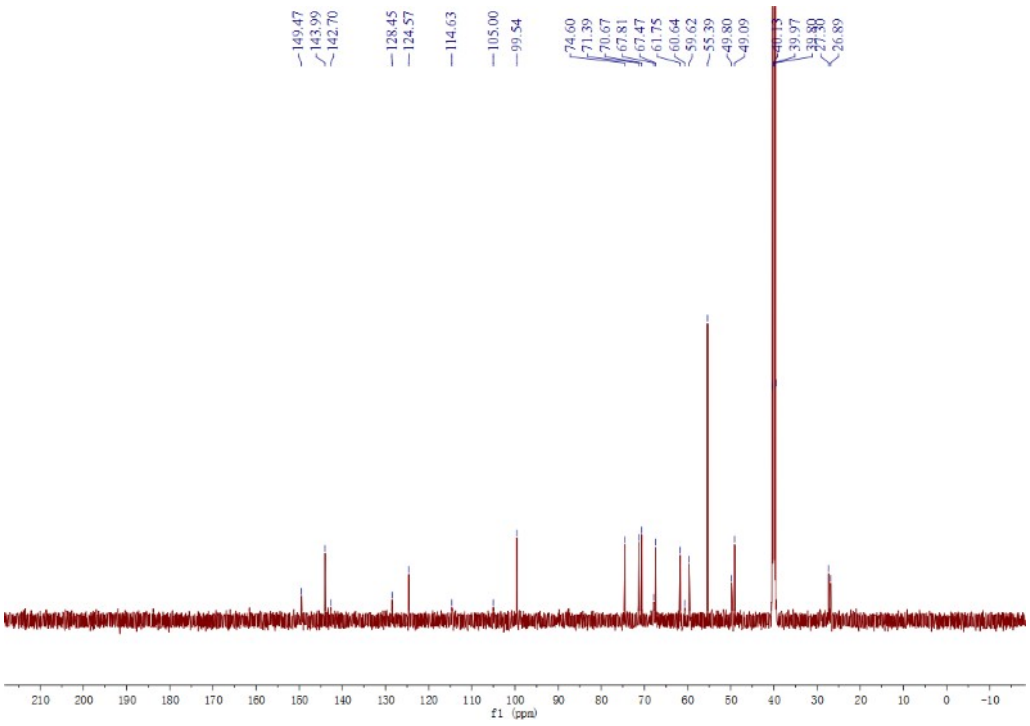


Fig. S9. ^{13}C NMR spectrum (125 MHz, DMSO-d_6, 298K) of compound **ManP5**

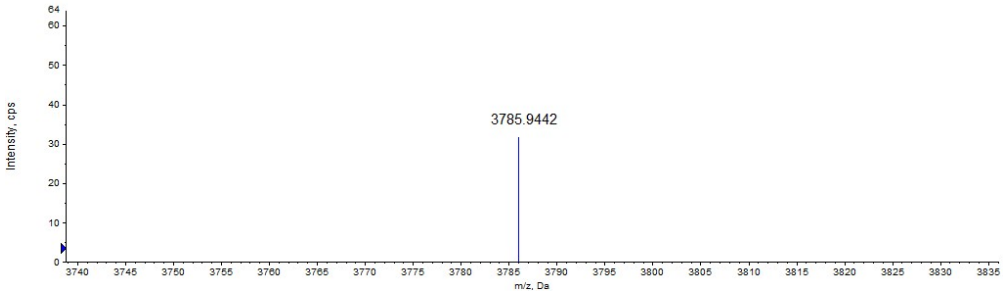
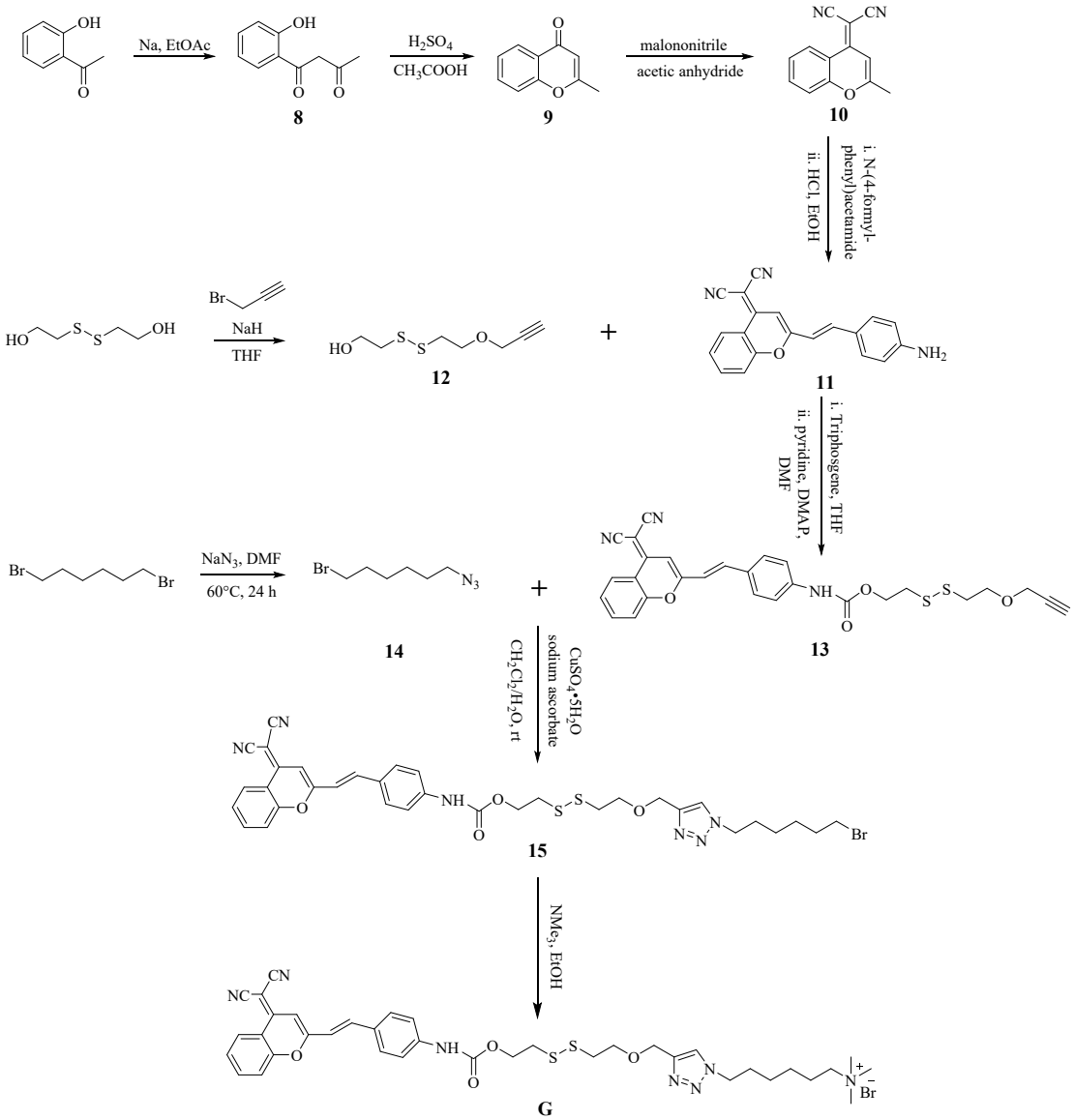


Fig. S10. ESI-MS spectrum of compound **ManP5**



Scheme S3. Synthetic route for dicyanomethylene-4H-pyran modified guest (**G**)

Synthesis of compound 8[S4]. 1-(2-hydroxyphenyl)ethanone (10.0 g, 73.5 mmol) was dissolved in 200 mL ethyl acetate, followed by adding sodium (8.00 g, 34 mmol). The grayish-green solid was filtered after violently stirring for 4 h at room temperature. The solid was dissolved in 100 mL methanol to consume the remained sodium. The solvent was evaporated under vacuum and the residue was dissolved in 100 mL deionized water, and then adjusted its pH to neutral. The aqueous solution was extracted with 200 mL ethyl acetate. The combined organic layers were dried over anhydrous $MgSO_4$. The filtrate was concentrated to yield the crude product of compound **8** as a brown solid, which was directly used in the next reaction without further purification.

Synthesis of compound 9[S4]. Sulfuric acid (4.6 mL) was slowly added to acetic acid glacial solution (70 mL) containing compound **8** (6.70 g, 37.5 mmol). The mixture was

refluxed for about 30 min and then was poured into 800 mL ice water, followed by adjusting its pH to neutral with Na_2CO_3. The aqueous solution was extracted with dichloromethane three times. The combined organic layers were dried over anhydrous $MgSO_4$. The filtrate was concentrated to yield the crude product of compound **9** as an acicular gray solid. The crude product was directly used in the next reaction without further purification.

Synthesis of compound 10[S4]. Compound 9 (4.60 g, 28.7 mmol) and malononitrile (2.40 g, 36.2 mmol) were dissolved in 25 mL acetic anhydride. The solution was refluxed for 14 h and then was evaporated under vacuum. Deionized water (80 mL) was added to the residue and the mixture was refluxed for another 0.5 h, followed by extraction with dichloromethane after cooling down to room temperature. The organic layers were combined and dried over anhydrous $MgSO_4$. The filtrate was concentrated to yield the crude product, which was purified by silica column chromatography to afford compound **10** as an orange solid (1.61 g, 27%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.92 (d, J = 8.8 Hz, 1H), 7.72 (td, J = 7.6, 1.4 Hz, 1H), 7.48-7.44 (m, 2H), 6.72 (s, 1H), 2.44 (s, 3H).

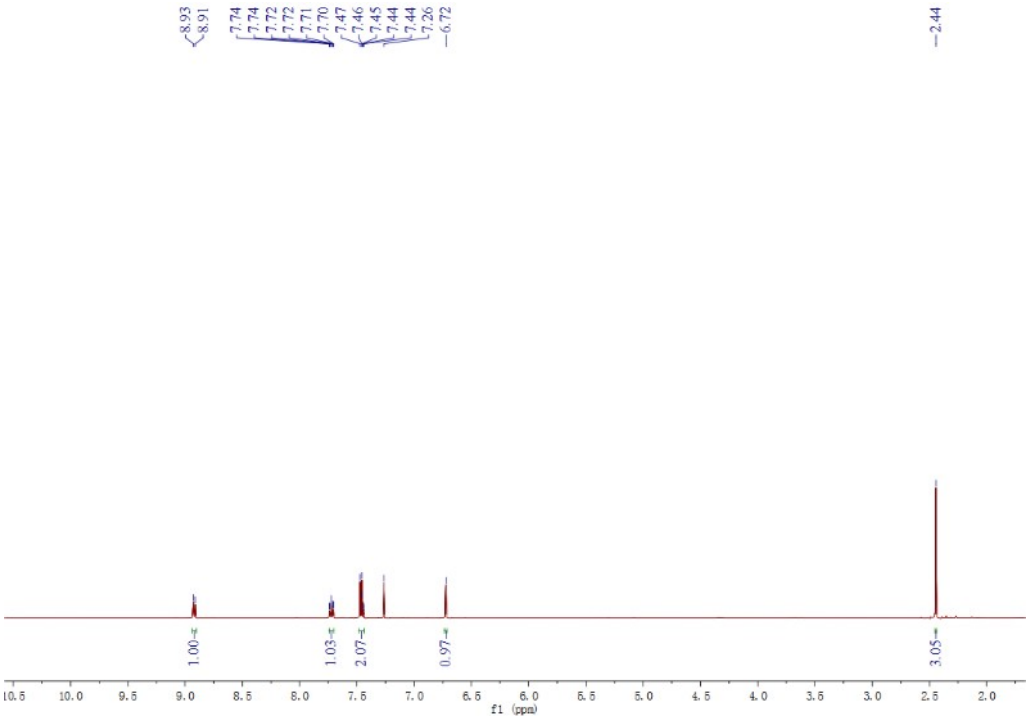


Fig. S11. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298K) of compound **10**

Synthesis of compound 11[S4]. Under argon atmosphere, 2-(2-methyl-4H-chromen-4-ylidene)malononitrile (200 mg, 0.95 mmol) and N-(4-formylphenyl)acetamide (142 mg, 0.88 mmol) were dissolved in toluene (10 mL) with piperidine (0.5 mL) and acetic

acid (0.5 mL) at room temperature. The mixture was refluxed for 12 h to give orange precipitate. After filtration, the orange solid was refluxed in a solution of conc. HCl and ethanol (2:1, v/v, 100 mL) for another 16 h. When the pH of solution was adjusted to neutral, the aqueous solution was extracted with ethyl acetate. The combined organic layers were dried over anhydrous $MgSO_4$. The filtrate was concentrated to yield the crude product, which was purified by silica column chromatography using petroleum ether/ethyl acetate (3:1, v/v) to yield compound **11** as a crimson solid (114 mg, 39%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.67 (d, J = 8.1 Hz, 1H), 7.84 (t, J = 7.7 Hz, 1H), 7.70 (d, J = 8.1 Hz, 1H), 7.65 (d, J = 8.7 Hz, 1H), 7.58 (d, J = 15.8 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.46 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 15.7 Hz, 1H), 6.79 (s, 1H), 6.61 (d, J = 8.4 Hz, 2H), 5.99 (s, 2H).

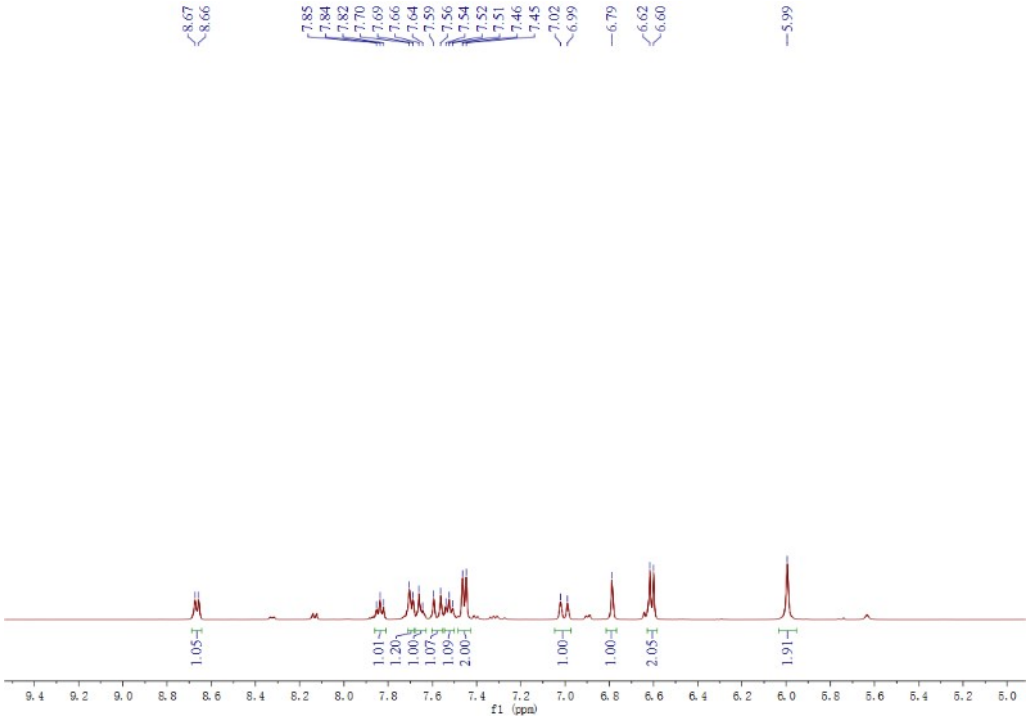


Fig. S12. ^{1}H NMR spectrum (500 MHz, DMSO-d_6, 298K) of compound **11**

Synthesis of compound 12[S4]. 2-hydroxyethyl disulfide (20.0 g, 130 mmol) and propargyl bromide (3.6 g, 65 mmol) were mixed in THF (200 mL). Under nitrogen atmosphere, NaH powder (3.0 g, 80 wt %, 98 mmol) was added into the reaction mixture in three batches within 3 h at 0 °C. The mixture was further stirred for another 7 h at room temperature before a few drops of water were added to stop the reaction. The mixture was filtered, and the solvent was removed by evaporation under reduced pressure at 50°C. The crude product was purified by silica column chromatography to give compound **12** as a pale-yellow clear oil (6.5 g, 52%). ^{1}H NMR (500 MHz, $CDCl_3$)

δ (ppm): 4.20 (d, J = 2.3 Hz, 2H), 3.90 (t, J = 5.5 Hz, 2H), 3.81 (t, J = 6.4 Hz, 2H), 2.93 (t, J = 6.4 Hz, 2H), 2.89 (t, J = 5.8 Hz, 2H), 2.47 (t, J = 2.3 Hz, 1H), 2.10 (s, 1H).

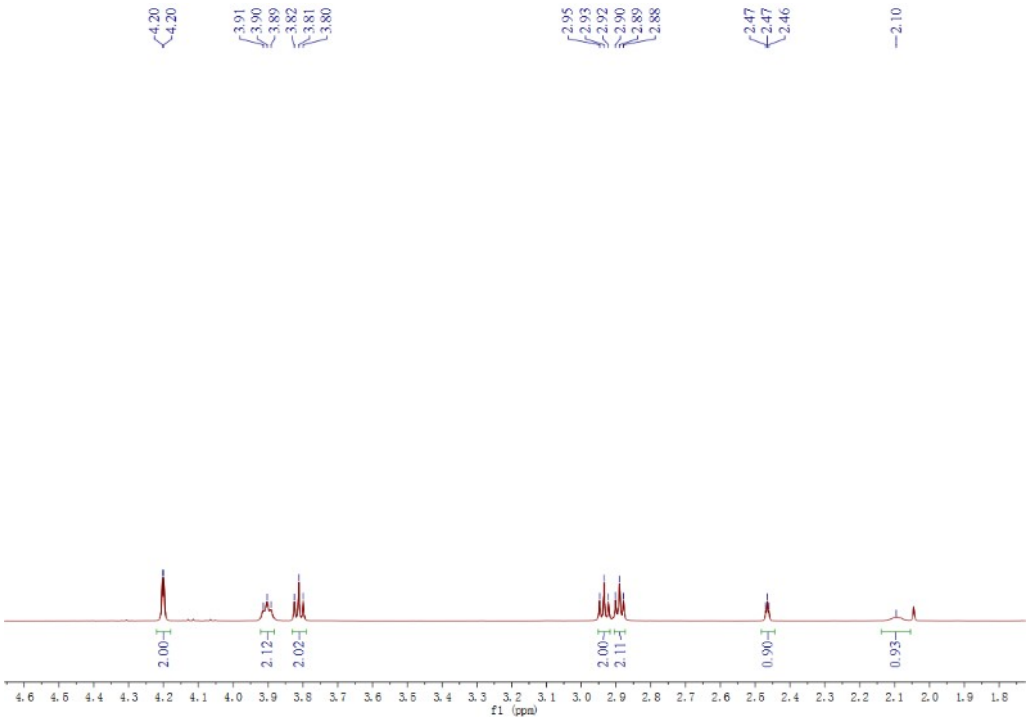


Fig. S13. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298K) of compound **12**

Synthesis of compound 13[S4]. Triphosgene (0.58 g, 1.87 mmol) was dissolved in dry THF (5 mL), then Compound **12** (0.30 g, 1.87 mmol) was added. The reaction solution was stirred for 12 h at room temperature. When the solvent was evaporated under vacuum, the residue was dripped into dry DMF (3 mL) containing pyridine (450 μL, 5.6 mmol), compound **11** (0.1 g, 0.28 mmol) and a catalytic amount (17 mg, 0.14 mmol) of DMAP. The solution was stirred for 14 h at room temperature, and the solvent was removed in vacuum. The pink residue was dissolved in CH_2Cl_2 (100 mL) and washed twice with 1 % HCl solution. The organic layer was dried over anhydrous $MgSO_4$, and the solvent was removed under vacuum again. The crude product was purified by silica column chromatography using petroleum ether/ethyl acetate (2:1, v/v) to afford compound **13** as a pink powder (114 mg, 67%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.05 (s, 1H), 8.74 (d, J = 7.2 Hz, 1H), 7.93 (t, J = 7.8 Hz, 1H), 7.80 (d, J = 8.6 Hz, 1H), 7.73-7.70 (m, 3H), 7.63-7.56 (m, 3H), 7.38 (d, J = 16.1 Hz, 1H), 7.00 (s, 1H), 4.36 (t, J = 6.2 Hz, 2H), 4.22 (s, 2H), 3.72 (t, J = 6.3 Hz, 2H), 3.44 (t, J = 2.4 Hz, 1H), 3.06 (t, J = 6.0 Hz, 2H), 2.98 (t, J = 6.3 Hz, 2H).

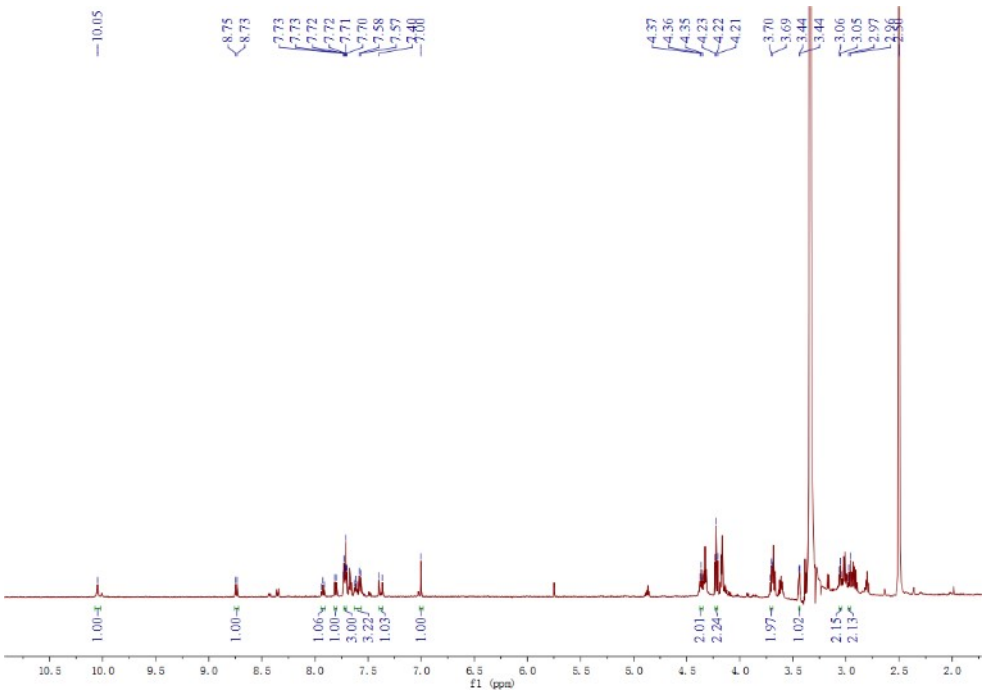


Fig. S14. ^{1}H NMR spectrum (500 MHz, DMSO-d_6, 298K) of compound **13**

Synthesis of compound 14[S5]. A solution of 1,6-dibromohexane (18.8 g, 76.9 mmol) and sodium azide (5 g, 76.9 mmol) in DMF (20 mL) with H_2O (5 mL) was stirred at 60°C for 24 h. The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL), dried with $MgSO_4$, and evaporated to dryness to give compound **14** as a pale-yellow oil (9.6 g, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.41 (t, J = 6.7 Hz, 2H), 3.28 (t, J = 6.9 Hz, 2H), 1.92-1.84 (m, 2H), 1.65-1.59 (m, 2H), 1.51-1.45 (m, 2H), 1.41 (m, 2H).

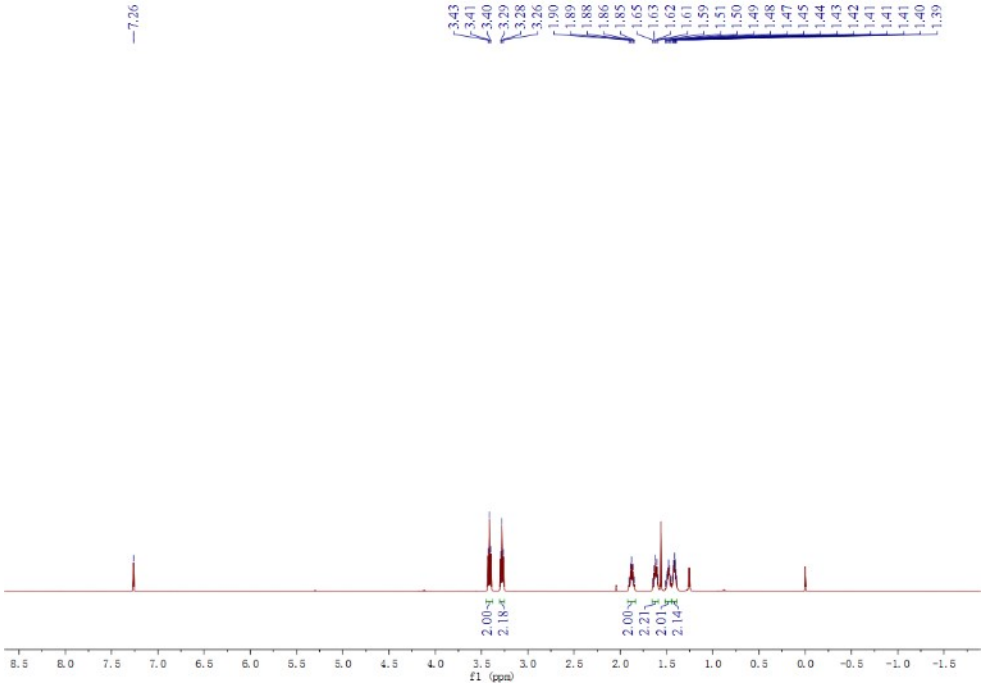


Fig. S15. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298K) of compound **14**

Synthesis of compound 15[S4]. Compound **13** (120 mg, 0.23 mmol), compound **14** (69.6 mg, 0.34 mmol) and copper sulfate pentahydrate (5.6 mg, 0.022 mmol) were dissolved in CH_2Cl_2/H_2O (1:1, 14 mL), and Sodium ascorbate (13.8 mg, 0.07 mmol) was added under nitrogen atmosphere. After the mixture was stirred for 24 h at room temperature, added water and the aqueous layer was extracted with dichloromethane. The organic layer was combined and dried over anhydrous sodium sulfate and filtered. The solvent was evaporated under vacuum. The obtained crude product was purified by column chromatography using petroleum ether/ethyl acetate (1:2, *v*/*v*) to afford compound **15** as a brownish red solid (57 mg, 34%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.05 (s, 1H), 8.74 (d, J = 8.3 Hz, 1H), 8.08 (s, 1H), 7.92 (t, J = 7.8 Hz, 1H), 7.80 (d, J = 8.2 Hz, 1H), 7.73-7.69 (m, 3H), 7.63-7.55 (m, 3H), 7.37 (d, J = 16.0 Hz, 1H), 6.99 (s, 1H), 4.54 (s, 2H), 4.33 (dd, J = 15.0, 6.8 Hz, 4H), 3.69 (t, J = 6.3 Hz, 2H), 3.48 (t, J = 6.7 Hz, 2H), 3.02 (t, J = 6.2 Hz, 2H), 2.95 (t, J = 6.3 Hz, 2H), 1.84-1.72 (m, 4H), 1.38 (dd, J = 15.0, 7.6 Hz, 2H), 1.23 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 158.98, 153.58, 153.41, 152.53, 144.12, 141.70, 139.03, 135.88, 129.72, 126.63, 125.12, 124.30, 119.55, 118.77, 118.09, 117.61, 116.44, 106.74, 68.14, 63.77, 62.72, 61.03, 60.18, 55.40, 50.99, 49.77, 49.68, 38.28, 37.22, 32.78, 30.25, 26.22, 25.99, 25.38. HRMS: m/z 759.1200 $[M + Na]^+$, 737.1374 $[M + H]^+$.

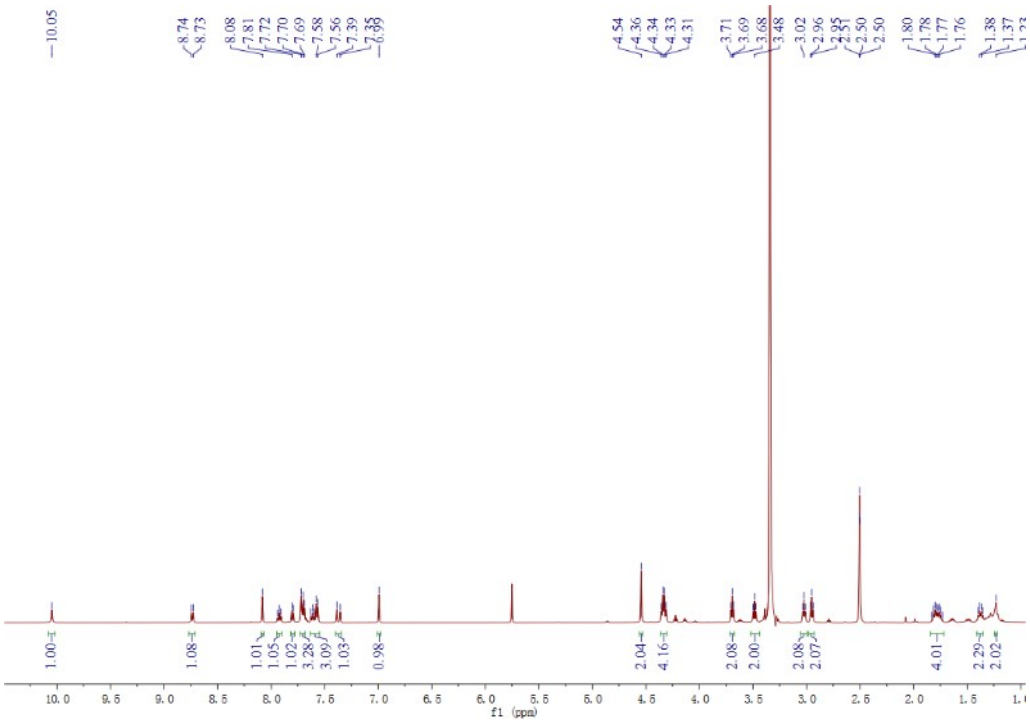


Fig. S16. ^{1}H NMR spectrum (500 MHz, DMSO-d_6, 298K) of compound **15**

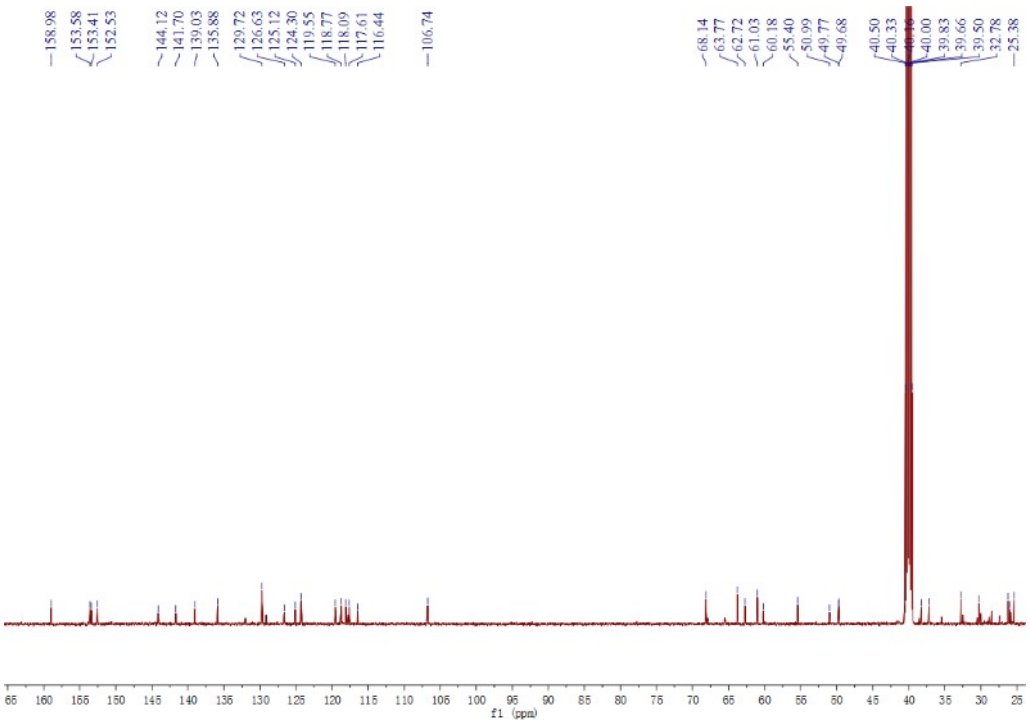


Fig. S17. ^{13}C NMR spectrum (125 MHz, DMSO-d_6, 298K) of compound **15**

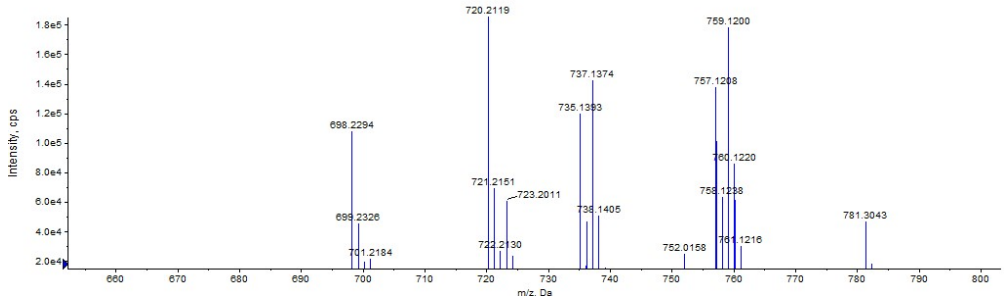


Fig. S18. ESI-MS spectrum of compound **15**

Synthesis of compound G[S6]. Compound **15** (147 mg, 0.2 mmol) and trimethylamine ethanol solution (33%, 0.7 g, 2.0 mmol) were dissolved in ethanol (15 mL) and stirred for 24 h. The solvent was evaporated under reduced pressure, recrystallized and dried in vacuo to give compound **G** as a brown solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.04 (s, 1H), 8.69 (d, J = 7.8 Hz, 1H), 8.10 (d, J = 12.2 Hz, 1H), 7.89 (d, J = 7.0 Hz, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 16.3 Hz, 3H), 7.56 (t, J = 8.8 Hz, 3H), 7.32 (d, J = 15.9 Hz, 1H), 6.93 (s, 1H), 4.54 (s, 2H), 4.34 (s, 4H), 3.70 (d, J = 5.2 Hz, 2H), 3.27 (s, 2H), 3.03 (s, 9H), 2.95 (s, 2H), 1.86-1.60 (m, 4H), 1.51-1.29 (m, 2H), 1.29-1.16 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 158.92, 153.56, 153.30, 152.48, 144.16, 144.12, 141.68, 138.99, 135.84, 129.70, 126.59, 125.09, 124.30, 119.51, 118.73, 118.04, 117.74, 117.56, 116.41, 106.69, 68.23, 68.15, 65.66, 63.81, 62.73, 52.64, 51.00, 49.63, 38.31, 37.22, 30.03, 29.88, 28.52, 25.99, 25.85, 25.63,

22.39. HRMS: m/z 714.2768 $[M - Br]^+$.

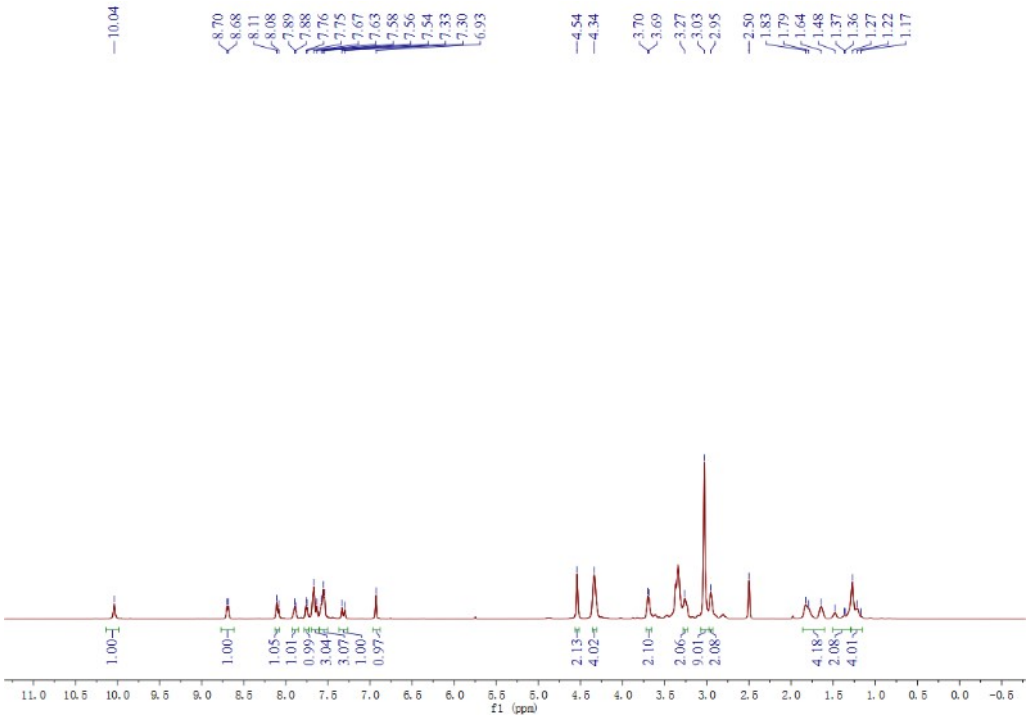


Fig. S19. ^{1}H NMR spectrum (500 MHz, DMSO-d_6, 298K) of compound **G**

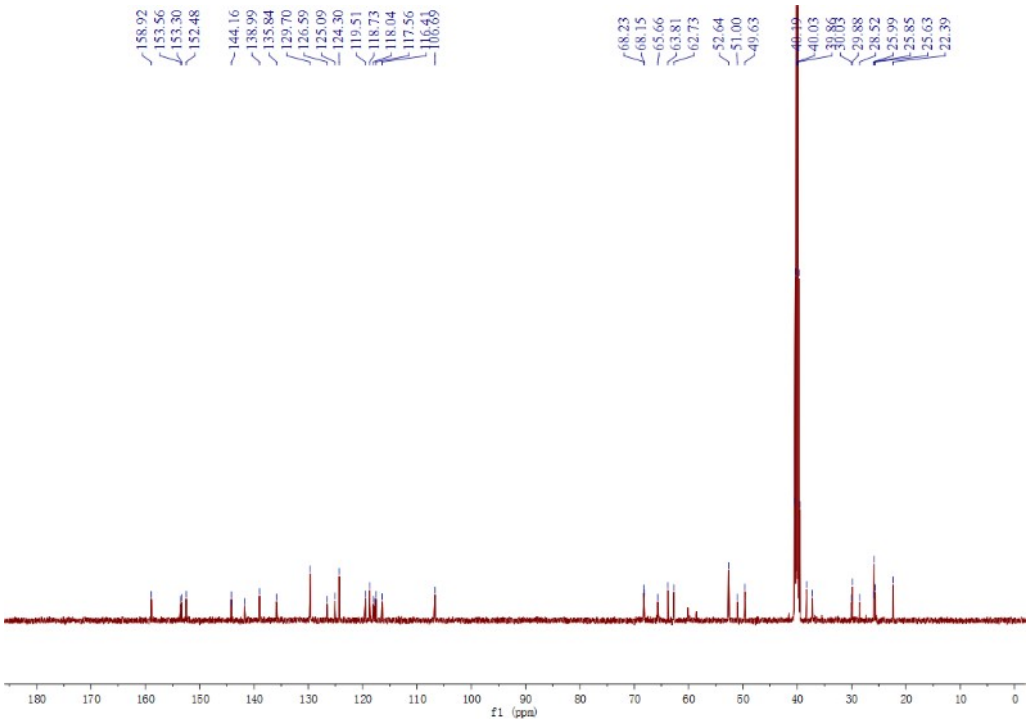


Fig. S20. ^{13}C NMR spectrum (125 MHz, DMSO-d_6, 298K) of compound **G**

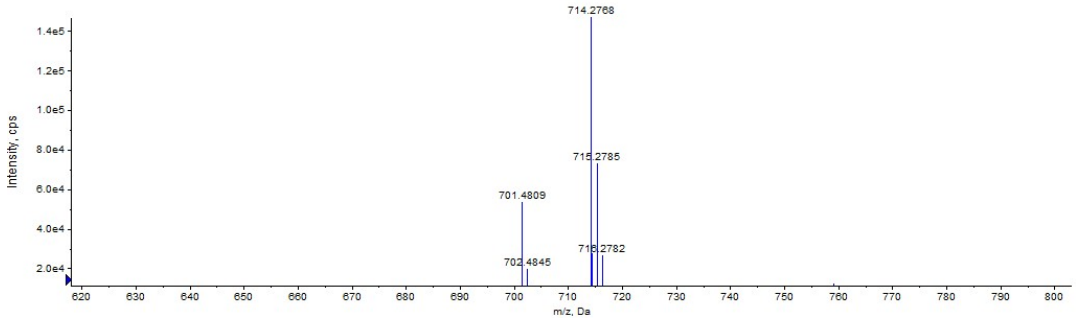


Fig. S21. ESI-MS spectrum of compound **G**

4. Investigation of the interaction between ManP5 and G

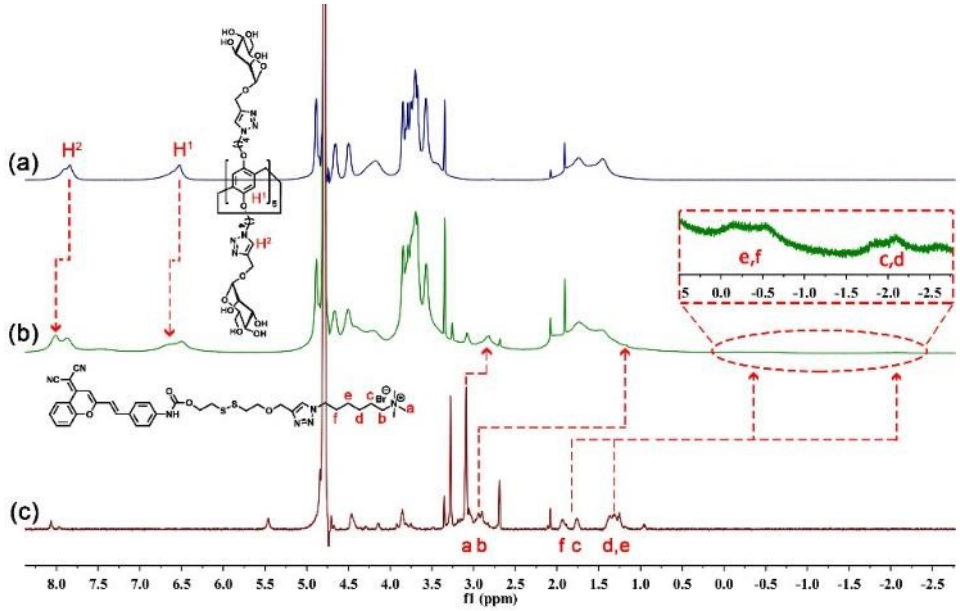


Fig. S22 ^{1}H NMR (500 MHz, D_2O, 298 K) spectra: (a) ManP5 (10.0 mM); (b) G (10.0 mM) and ManP5(10.0 mM); (c) G (10.0 mM).

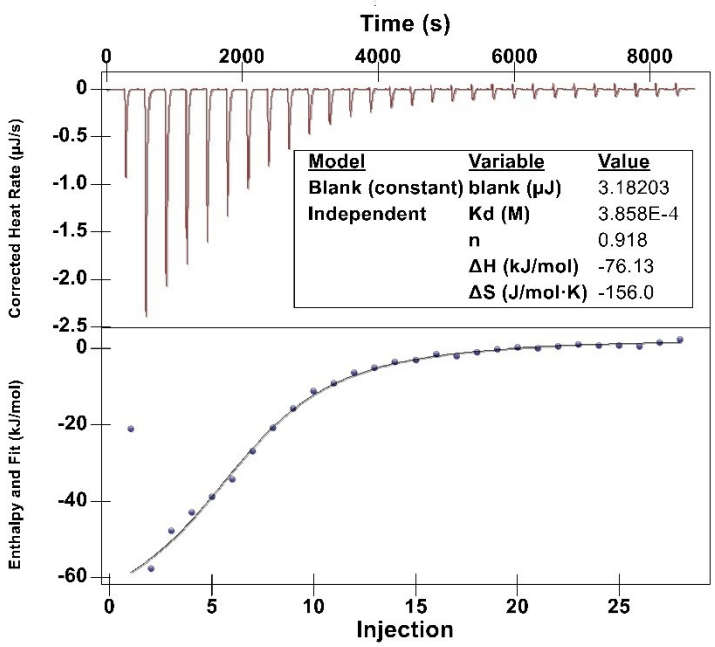


Fig. S23 Microcalorimetric titration of ManP5 and G in D.I. water at 298.15K. (TOP) Raw ITC data for 28 sequential injections (3.54 µL per injection) of a ManP5 solution (1.00 mM) into a G solution (0.10 mM). (Bottom) Net reaction heat obtained from the integration of the calorimetric traces.

5. Fabrication of ManP5⊃G supramolecular vesicles

(1) Compound G (1.4 mg, 0.002 mmol) was added to a solution of ManP5 (15.1 mg, 0.002 mmol) in 4 mL of sterile water. The mixture was subjected to ultrasonication for 30 min, and then left to stand still overnight to obtain the vesicles, which were characterized by DLS, SEM and TEM, respectively.

(2) In order to verify the stability of the ManP5⊃G NPs, the obtained vesicles were added into PBS (pH = 7.4), PBS (pH = 6.5), DMEM medium and DMEM medium containing 10% FBS, and the average particle size were detected by DLS after 24 h.

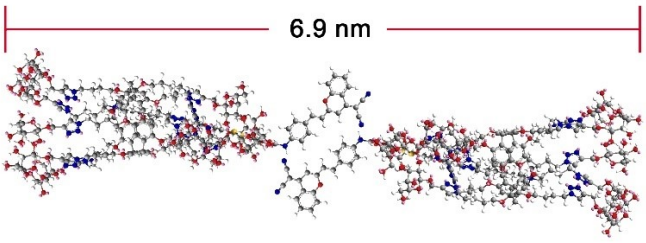


Fig. S24 The energy-minimized structure of ManP5⊃G (ball and stick mode).

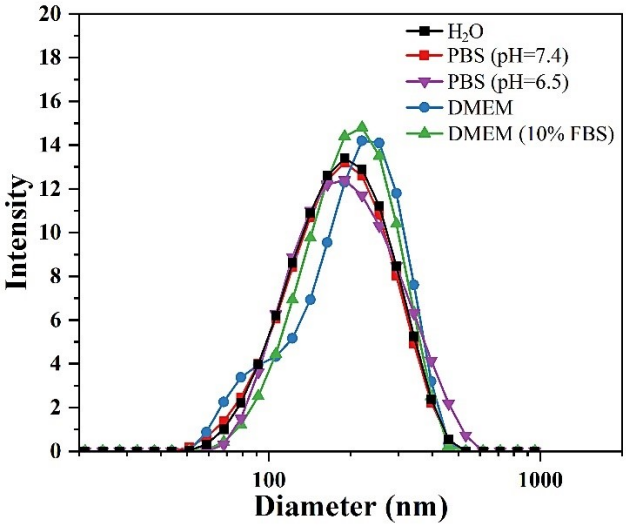


Fig. S25 The average size distribution of ManP5⊃G NPs in different conditions.

6. GEM loading and drug release behavior.

The aqueous solution of GEM and ManP5⊃G was added to 1.041 mL of ultrapure water, then agitated the mixture for 24 h. After that, the unloaded GEM was removed by dialysis (molecular weight cut-off (MWCO) = 2,000 Da) against distilled water for 24 h to obtain GEM@ ManP5⊃G nanoparticles.

The release profile was further evaluated by dialysis under different concentration of GSH conditions by mimicking the microenvironment in normal tissues or pathological tissues/endosomal compartments. The concentration of GEM was determined by measurement of absorbance at 268 nm using a standard absorbance vs. concentration curve constructed for GEM in the corresponding release medium.

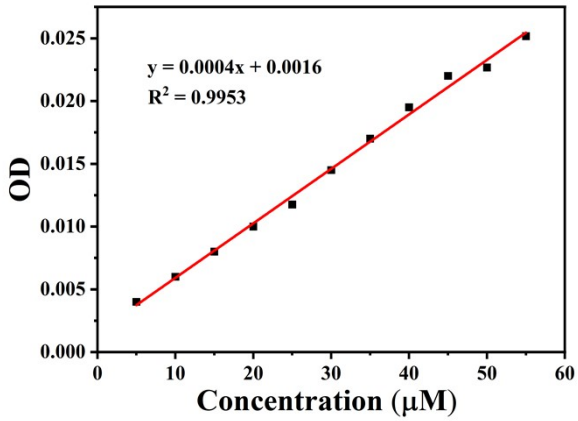


Fig. S26. GEM standard curve at 268 nm

7. Cell culture

Human breast cancer cells MCF-7, human liver cells HL7702, and human renal epithelial cells 293T were obtained from KeyGEN BioTECH Co. (Nanjing, China). HL7702 and 293T cell lines were cultured in 1% (v/v) antibiotics and 10% (v/v) FBS supplemented Roswell Park Memorial Institute medium (RPMI 1640, Gibco) under 5% CO_2 at 37 °C. MCF-7 cells were cultured at the same conditions except Dulbecco's Modified Eagle Medium (DMEM, Gibco) was used.

8. Cellular uptake and targetability of ManP5⊃G

MCF-7 cells were seeded onto confocal dish with 1 x 10^5 cells per dish. After 24 h culturing, cells were transferred to fresh medium containing 10 μM ManP5 ⊃G and continue culture for different time. Cells were washed with PBS before using confocal laser scanning microscope (CLSM) and flow cytometry analysis to detect cell uptake.

The internalization pathway of ManP5⊃G NPs was investigated where chlorpromazine (Chl), genistein (Gen), and Mannose (Man) were commonly used to inhibit clathrin-mediated endocytosis, caveolae-mediated endocytosis, and mannose receptor-mediated endocytosis respectively. In addition, 4 ℃ environment as an inhibitory condition for reducing cells membrane fluidity. Cells were treated with different inhibitors (Chl, Gen, or Man) for 4 h before ManP5⊃G NPs were added and measured by flow cytometry. The intracellular colocalization of ManP5⊃G NPs was measured by CLSM.

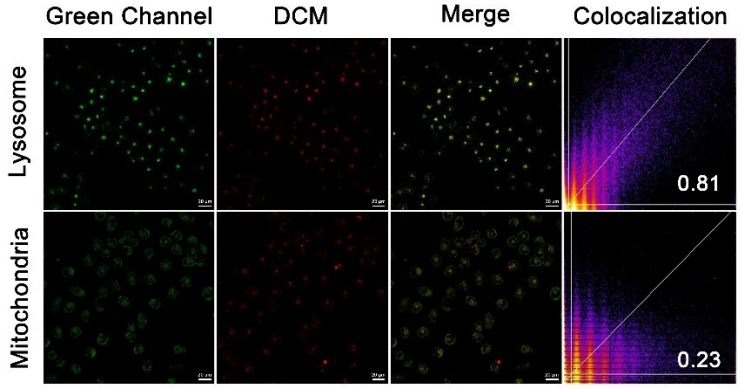


Fig. S27 Colocalization of ManP5⊃G NPs with the lysosome and mitochondria were detected *via* CLSM. The scale bar is 20 μm.

9. Real-time monitoring of intracellular drug release

In order to evaluate the ability of ManP5⊃G NPs to monitor drug delivery and

release in real time, sodium fluorescein (NaFL) was loaded into nanoparticles as a substitute for the non-fluorescent drug GEM. The NaFL-loaded vesicles were cultured with MCF-7 cells for 2 h and 4 h and observed by CLSM, respectively.

10. Biocompatibility of ManP5⊃G NPs

293T cells were seeded onto 96-well plate (5×10^3 cells/well), respectively. After overnight culture, cells were treated with ManP5⊃G NPs with different concentrations for 24 h, 48 h and 72 h, respectively. And the MTT assay was used to detect cell viability.

11. Cell cytotoxicity of GEM@ManP5⊃G NPs

HL7702, 293T, MCF-7 cells were seeded onto 96-well plate (5×10^3 cells/well), respectively. After overnight culture, cells were treated with free GEM or GEM@ ManP5⊃G (doses of GEM@ManP5⊃G: 0.1, 0.2, 0.5, 1, 2 μM in medium) for 48 h or 72 h, respectively. and the MTT assay was used to detect cell viability.

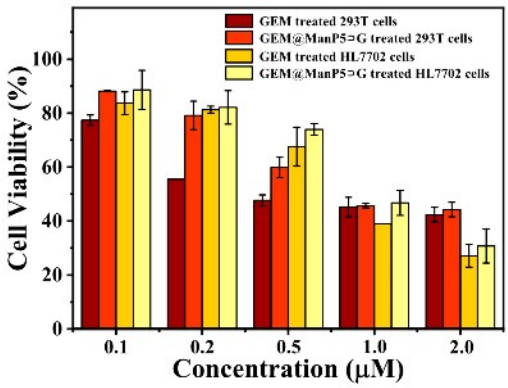


Fig. S28 The cytotoxicity results of GEM and GEM@ManP5⊃G NPs on 293T and HL7702 cells for 48 h

12. References

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